PRESTOCKING ASSESSMENT OF THE PREVALENCE AND INTENSITY OF DIPHYLLOBOTHRIUM DITREMUM (CREPLIN) PLEROCERCOIDS IN FRESHWATER BARRIERED LAKES IN ALASKA

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A

THESIS

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By
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Juneau, Alaska
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ABSTRACT

Plerocercoids of the pseudophyllidean cestode Diphyllobothrium ditremum (Creplin, 1825) have significantly affected the success of using certain barriered lakes for the rearing, overwintering and smolting of juvenile coho (Oncorhynchus kisutch) and chinook (Oncorhynchus tshawytscha) salmon by causing mass mortalities of these host fishes. The prevalence of cestode procercoids in copepods, the first intermediate host, was propose as a method for assessing the potential for cestode caused losses of salmon prior to stocking a lake. However, no procercoids were found in a total of 15,276 Diaptomus and 435 Cyclops spp. from three lakes on south Baranof Island examined for procercoids.

Diaptomus kenai is suggested as the first intermediate host for Diphyllobothrium ditremum, despite the absence of procercoids in any specimens examined. Diaptomus kenai was the predominant copepod in the three lakes studied, and was the prey item occurring most frequently (percent occurrence, 73.7%) in the stomach contents of 95 resident coho. Coho, chinook, and rainbow trout (Salmo gairdneri) were obvious second intermediate hosts of D. ditremum. Among three species of piscivorous birds examined from the lake sites, a single common merganser (Mergus merganser) contained seven mature worms resembling D. ditremum.

A bioassay study using coho salmon fingerlings in net pens suspended within a "cestode infested" lake proved successful as an assessment method. Plerocercoids of D. ditremum were observed in 91% of the planted coho within twenty days of exposure in Osprey Lake. Coho mortalities of 46.2% and 22.4% were observed in two pens. Mean plerocercoid intensities for apparently normal, moribund, and dead coho were 11, 28, and 32 respectively. Moribund and dead coho each had significantly larger worm loads than apparently normal coho.

Primary lesions observed from gross and histopathological examinations of parasitized coho from Elfendahl and Osprey lakes included: ascites with marked distension of the abdomen; hemorrhaging of viscera primarily adipose tissue and liver; and focal necrosis of organs from migrating plerocercoids.

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INTRODUCTION

Plerocercoids of the pseudophyllidean cestode Diphyllobothrium are significant salmonid pathogens capable of causing severe disease and fish mortality in natural bodies of water (Simms and Shaw 1931; Duguid and Sheppard 1944; Hickey and Harris 1947; Fraser 1960; Becker 1966; Becker and Brunson 1967; Hoffman 1967; Bylund 1972). In Alaska a recent method of salmonid rearing has been the use of one-way barriered lakes for the fry-to-smolt rearing of coho (Oncorhynchus kisutch) and chinook (Oncorhynchus tshawytscha) salmon. The Northern Southeast Regional Aquaculture Association (NSRAA) and the Fisheries Rehabilitation, Enhancement, and Development (FRED) Division of Alaska's Department of Fish and Game (ADF&G) are two agencies that have successfully used such lakes for rearing salmonids. However, parasitism of stocked fingerlings by Diphyllobothrium sp. plerocercoids has limited the success of this method in certain lakes.

In late June 1983, 115,300 coho were stocked in Elfendahl Lake on Chichagof Island by NSRAA. At least half of these fish were expected to emigrate as smolts, but largely due to Diphyllobothrium parasitism only 7% survived to enter sea water (R. A. Crone, pers. comm., NSRAA, Sitka). In June 1984, NSRAA and the National Marine Fisheries Service (NMFS) planted 141,500 chinook into Osprey Lake on Baranof Island. By August numerous chinook mortalities had been observed associated with Diphyllobothrium in 80%

of the fish examined (T.R. Meyers, pers. comm., FRED, Juneau). Less than 18% of the transplanted chinook survived to enter sea water (R. Heintz, pers. comm., NMFS, Juneau).

Other lakes on Baranof Island, including Banner, Tranquil, Larry, Ludvik, and Lower Rostislaf have been stocked without known cestode-caused mortality. Osprey Lake was first stocked with salmon in 1975 when 276,000 coho fry were introduced. In contrast to the recent chinook fry transplant, these coho did not suffer losses from cestode infestation and half of them emigrated as yearling smolts (Crone 1981). Because of the variable success of stocking salmon fry in barriered lakes, early detection of problem lakes prior to stocking has become a necessity if the lake rearing program is to be continued by NSRAA and the FRED Division.

The purpose of this study was to develop a method for detecting lakes with a potential cestode problem. Criteria for defining potential problem lakes first required quantification of plerocercoid numbers of D. ditremum (Creplin 1825) causing morbidity. Halvorsen (1970) and Henricson (1977, 1978) did not consider D. ditremum to be important in parasite induced host mortality. Plerocercoids of D. dendriticum and D. ditremum often occur together (Wootten and Smith 1979), but D. ditremum is smaller in size and does not have the same ability to migrate in the host as D. dendriticum (Halvorsen 1970; Halvorsen and Wissler 1973). Hickey and Harris (1947) also suggested that it was D. dendriticum and not D. ditremum that caused mortality in trout.

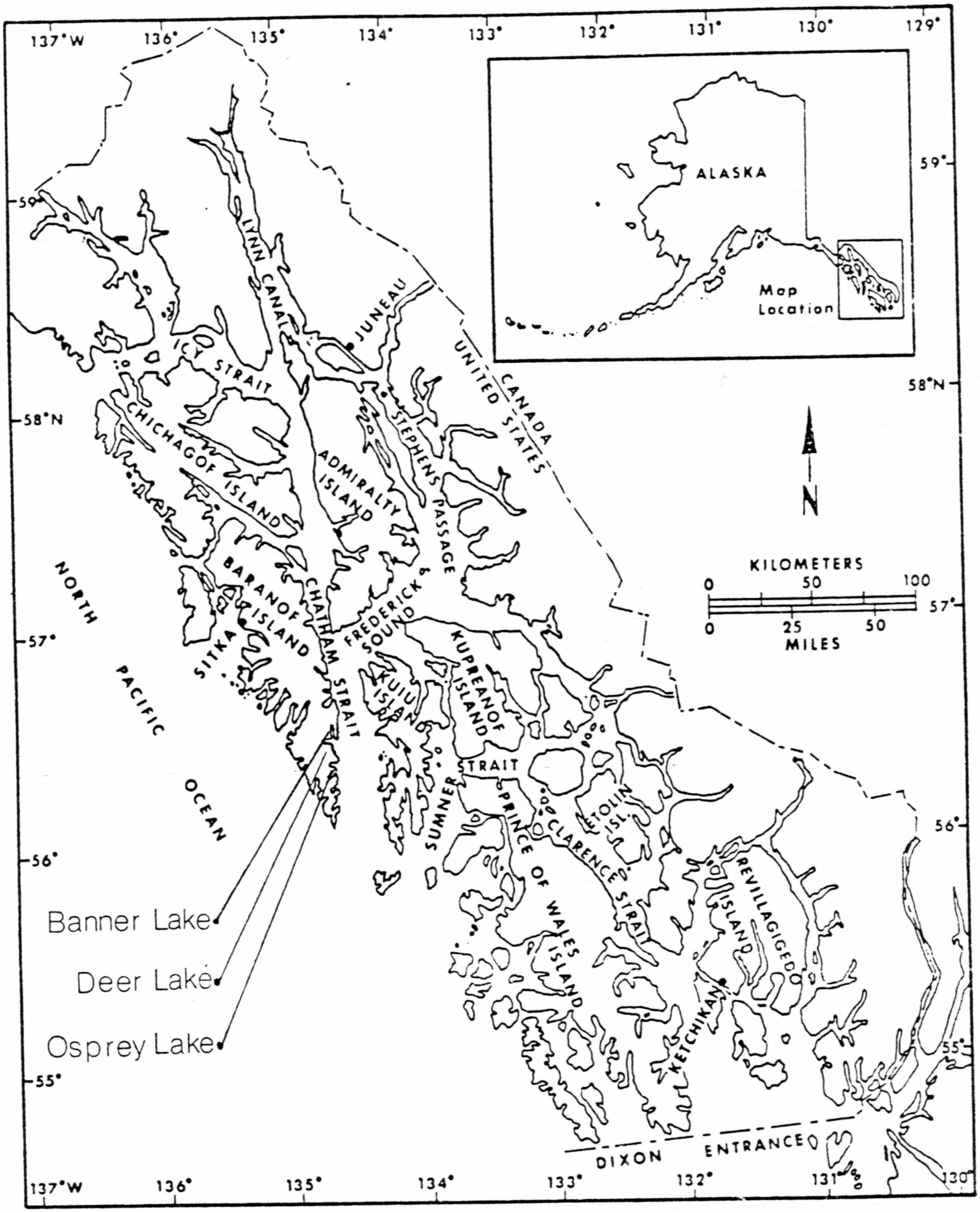
Only recently was D. ditremum suggested as a cause of mortality in intermediate host populations of fish (Halvorsen and Anderson 1984). This study provides new information regarding the lethal effects of D. ditremum. By utilizing this information and the methods proposed for detecting the prevalence and intensities of plerocercoids, future epizootics of salmonids in lake rearing programs may be prevented. The continued success of lake rearing salmonids will ultimately benefit Alaska's economy.

GENERAL EXPERIMENTAL DESIGN

Zooplankton were periodically collected from Deer, Banner, and Osprey lakes on Baranof Island south of the city of Sitka (Figure 1). The copepod portion of subsamples were carefully examined for procercoids according to methods used by Watson and Lawler (1965). Copepods are well documented as first intermediate hosts of diphyllobothriids (Wardle 1935; Duguid and Sheppard 1944; Chandler and Clark 1961; Dogiel et al. 1961; Guttowa 1961, 1963; Meyer and Vik 1963; Halvorsen 1970; Johnson 1975; Dau and Barrett 1981). Consequently, I hypothesized that the prevalence of procercoids in copepods could be correlated to the resultant prevalence and intensity of plerocercoids in planted salmonids during a growing season. Concurrently with zooplankton collections, salmonids were collected and examined for plerocercoids.

Because of the Diphyllobothrium epizootic that occurred at Osprey Lake in the previous year, a bioassay study using coho fingerlings in net pens suspended within the lake was initiated. Necropsies and histopathological examinations were preformed on these fish to ascertain any lethal effects of Diphyllobothrium plerocercoids.

Figure 1: Banner, Deer, and Osprey lakes in Southeastern Alaska. (Adapted from Crone 1981)

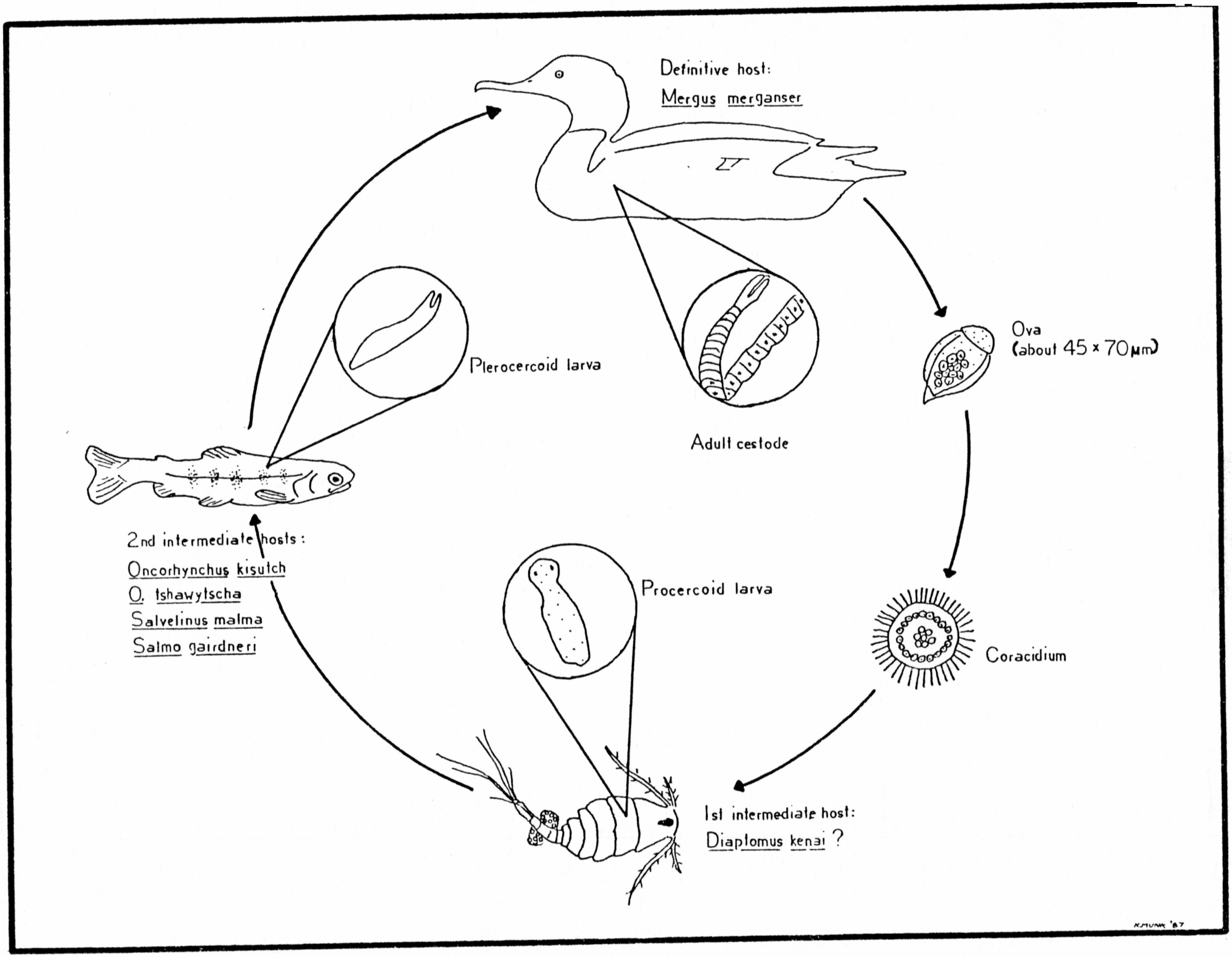

137°W
136°
135°
134°
133°
132°
131°
130°
129°
59°
58°N
57°
56°
55°
ALASKA
Map Location
LYNN CANAL
JUNEAU
ICY STRAIT
CHICHAGOF ISLAND
ADMIRALTY ISLAND
STEPHENS PASSAGE
UNITED STATES
CANADA
BARANOF ISLAND
CHATHAM STRAIT
FREDERICK SOUND
SITKA
KUIU ISLAND
KUPREANOF ISLAND
NORTH PACIFIC OCEAN
SUMNER STRAIT
PRINCE OF WALES ISLAND
CLARENCE STRAIT
ETOLIN ISL.
REVILLAGIGEDO ISLAND
KETCHIKAN
DIXON ENTRANCE
KILOMETERS
0 50 100
0 25 50
MILES
N
Banner Lake
Deer Lake
Osprey Lake

ASSESSMENT OF THE PREVALENCE, INTENSITY, AND LETHALITY OF DIPHYLLOBOTHRIUM LARVAE IN COPEPODS AND SALMONIDS FROM THREE ALASKAN LAKES

Introduction

The general life cycle of *Diphyllobothrium* sp. is illustrated in Figure 2. Adult worms are usually found in the small intestine of piscivorous birds such as those of the genera *Larus*, *Mergus*, *Gavia*, and *Phalacrocorax* (Vik 1964). Records in North America indicate that gulls (*Larus* spp.) are the most common definitive host (Becker and Brunson 1967). Infested birds defecating in freshwater lakes deposit demersal eggs. Hatching time depends on water temperature and species but for most diphyllobothriids it takes 6-19 days at 20°C (Hillard 1960). Hatching time increases as water temperature decreases. After eclosion, free-swimming coracidia emerge which must be eaten by copepods within 1-3 days or the cestode larvae die (Hillard 1960; Chandler and Clark 1961; Meyer and Olsen 1975). In copepods, parasite larvae penetrate the gut and become procercoids in the host's hemocoel. Complete procercoid differentiation occurs in approximately 2 weeks (Rosen and Dick 1983a). Procercoid transmission to salmon occurs as a result of fingerlings eating infested copepods. Procercoids penetrate the salmon gut and become

Figure 2: Life cycle of Diphyllobothrium ditremum.


Definitive host:
Mergus merganser
Ova
(about 45 × 70 µm)
Adult cestode
Plerocercoid larva
Coracidium
Procercoid larva
1st intermediate host:
Diaptomus kenai ?
2nd intermediate hosts:
Oncorhynchus kisutch
O. tshawytscha
Salvelinus malma
Salmo gairdneri
K.MUNK '87

plerocercoids that migrate to host tissues or the body cavity. A plerocercoid has a primordial scolex that resembles the adult structure. Plerocercoids may grow to length of several centimeters. The life cycle is completed when a definitive host eats an infested fish.

Mass mortalities of salmonids infested with Diphyllobothrium plerocercoids have been reported by several authors (Simms and Shaw 1931; Duguid and Sheppard 1944; Hickey and Harris 1947; Fraser 1960; Hoffman and Dunbar 1961; Becker and Brunson 1967). In Southeast Alaska, two recent Diphyllobothrium epizootics of planted coho and chinook fry in barriered lakes have created a need for the early detection of lakes with a cestode threat. Coho fry transplanted into Lake Elfendahl on Chichagof Island in late June 1983 (mean size = 1.0 g) sustained only a 7% survival to enter sea water due to Diphyllobothrium plerocercoid infestation. Diphyllobothrium plerocercoids were also responsible for only an 18% survival to sea water of Chinook fry transplanted into Osprey Lake in June 1984 (mean size = 1.9 g). The objectives of this study were fourfold: (1) to determine the prevalence of procercoids in copepods and correlate these data with the resultant prevalence and intensity of plerocercoids in salmonids, (2) to identify the species of Diphyllobothrium based upon retrieval of adult worms from suspected hosts, (3) to determine the lethal intensity of plerocercoids in stocked salmon fry, and (4) to determine a method of identifying lakes with a cestode threat prior to stocking.

Materials and Methods

Deer, Banner, and Osprey lakes are located on the southern end of Baranof Island approximately 80 km south of the city of Sitka (Figure 1). Each lake is situated above an outlet barrier falls that prevents fish from entering, and all are within a few kilometers of each other.

Banner Lake, which is fishless, was successfully stocked in 1982 with 97,500 coho (mean size = 1.2 g) by the Northern Southeast Regional Aquaculture Association (NSRAA). Consequently, it was hypothesized that Banner Lake would be at low risk for Diphyllobothrium parasitism, and it could serve as the control lake in this study. Banner Lake was stocked on 18 July 1985 with 96,100 chinook fry (mean size = 1.0 g) supplied by the National Marine Fisheries Service (NMFS). Deer Lake was chosen as the experimental lake in this study because it has resident rainbow trout (Salmo gairdneri) that are parasitized by Diphyllobothrium plerocercoids (T. R. Meyers, pers.comm., FRED Division, ADF&G). Deer Lake was stocked by NSRAA on 1 and 2 July, 1985 with approximately 780,800 coho fry (mean size = 0.8 g). It was expected that these coho would become parasitized by Diphyllobothrium sp.. Additionally, 657 coho from the same group that was transplanted into Deer

Lake were placed in three net pens suspended within Osprey Lake on 4 July, 1985. Because of the Diphyllobothrium epizootic that occurred there the year before, it was expected that these coho would also become parasitized by Diphyllobothrium sp..

The impact of using two species of Oncorhynchus of roughly the same size and age is assumed to be negligible. Both coho and chinook salmon were similarly affected by Diphyllobothrium parasitism in the Elfendahl and Osprey Lake epizootics.

Methods similar to those used by Watson and Lawler (1965) for collecting and examining copepods, were incorporated into this study. Zooplankton samples were collected by making 100-m oblique surface tows with a 153-μm mesh net in about 1 m of water. Samples were taken at approximate semimonthly intervals from April through October 1985 at Deer Lake and from May through October 1985 at Osprey and Banner lakes. Four stations were established at each of Deer and Banner lakes, and three were established at Osprey Lake (Appendix Figures A1, A2, A3). Two tows made at each station were subsampled twice as 1-2 ml aliquots. Most of the time zooplankters were narcotized with ethanol (ETOH) so that live animals could be more easily examined and procercoids more readily detected (Watson and Lawler 1965; Henricson 1978). Alternatively, samples were preserved in 70% ETOH and examined at a later date. Copepods were examined, identified according to Pennak (1978), and counted under a stereomicroscope.

Birds of the genus Larus are natural definitive hosts of diphyllobothriids (Vik 1964; Dau and Barrett 1981). In the spring of each year mew gulls (Larus canus) regularly use freshwater lakes in Alaska as nesting grounds. The proximity of these birds should increase the possibilities for collecting copepods parasitized by procercoids. Therefore, at least one zooplankton sampling station in each lake was established near an active mew gull nest (Appendix Figures A1, A2, A3).

A total of 37 rainbow trout were collected near the zooplankton sampling stations on Deer Lake between 24 April and 24 July, 1985. A sinking variable-mesh gill net was used to capture these fish. The trout were killed with a blow to the head for immediate necropsy or placed on ice and examined within 24 hours. Plerocercoids were placed in petri dishes filled with distilled water and grouped by their location in a particular organ or tissue. All worms were counted but only fully relaxed specimens were measured to the nearest 0.25 mm. Trout were measured for fork length and scales collected for age determination.

After stocking Deer and Banner lakes, 30 salmon from each lake were sampled concurrently with zooplankton collections. Two additional salmon samples were collected from Deer Lake on 21 November, 1985 and 24 May, 1986. Three-foot long, vexar funnel traps baited with salmon eggs were used to capture the fish. Approximately 8 fish were collected near each zooplankton station. Fish were anesthetized in a concentrated solution of tricain methane sulfonate (MS-222) before measuring fork lengths, flushing their stomachs, and preforming necropsies. Stomachs were flushed using a 10-cc syringe and a blunted 25 gauge hypodermic needle. Contents were preserved in 70% ETOH. Fish that were not examined immediately were put on ice for later necropsy the following day. Plerocercoids were processed as discussed above.

On 4 July, 1985, three 3-mm mesh, 4-m x 4-m x 6-m deep net pens were positioned within Osprey Lake (Appendix Figure A3) Each pen frame consisted of four 5-m long cedar poles that were lashed together to form a 4.6 m square. A large buoy was lashed to each corner, and a net was nailed and tied to each frame. The opening of each pen floated approximately 0.5 m above the water surface. Nets were weighted at the bottom with a lead line to maintain a box shape. Each pen was secured in position with an anchor and a line tied to shore. Pens A and B were placed in the west basin approximately 15 m off the south and north shores, respectively. Pen C was located in the east basin approximately 25 m off the north shore.

Two hundred and nineteen coho were place in each pen on 4 July and left to feed only on natural food items from the ambient water. Stocking size was 47 mm (mean fork length). Coho were sampled from the net pens at approximately 1, 3, 5, 7, 9, and 15 weeks post-stocking to gather data on size, food consumption, mortality, and plerocercoid prevalence and intensities. Coho were categorized into one of three groups: nonmoribund (apparently normal), moribund, or mortality. Those that had ascites were classified as moribund.

Fish and plerocercoids were processed using methods previously discussed. Decomposing mortalities were excluded from fish samples because plerocercoids likely had left these fish.

All of the fish in Pen B escaped to the lake sometime between 26 July and 4 August when high water caused the collapse of one of the corners. Metal poles were substituted for the cedar poles on the two remaining pens.

Approximately 78 coho from Pen A also escaped into the lake sometime between 9 September and 19 October when a large drifting log tore part of the net from the frame. Some of the escaped fish were recaptured with baited funnel traps on 8, 23, 29, August and on 21, 22, 24 October, 1985.

Six mew gulls, Larus canus, one merganser, Mergus merganser, and one belted kingfisher, Ceryle alcyon, were collected, by shooting them, at Deer and Osprey lakes during April and May, 1985 and in May, 1986 and examined for adult cestodes. In addition, infection experiments were carried out with two 15-day-old chickens using plerocercoids obtained by necropsy of Deer Lake rainbow trout and coho. One chicken was fed 6 plerocercoids on 10 April, 1986 and 15 additional worms on 24 May. A second chicken was fed 16 plerocercoids on 24 May. The chickens were fed by means of a 10-cc syringe containing tap water and the plerocercoids. Feces were checked for cestode eggs on 25 April, 31 May, and 16 June. Both chickens were killed for necropsy on 17 June. Statistical analyses of data were accomplished using methods outlined in Sokal and Rohlf (1981).

Results

Procercoid Prevalence:

The calanoid copepod Diaptomus kenai was the most abundant zooplankton in Deer, Banner, and Osprey lakes. The cyclopoid copepod, Cyclops vernalis, was also present but much less abundant. Totals of 13,426 Diaptomus sp. and 374 Cyclops sp. were examined from these lakes with no procercoids found. At Osprey Lake stomach samples of 1,709 Diaptomus sp. and 61 Cyclops sp. from 95 coho were also without procercoids (Table 1). Diaptomus occurred in 70 of the 95 stomachs (percentage occurrence 73.7) and more than 100 Diaptomus per stomach were found in 7 fish. The cladoceran Eubosmina appeared in high abundance (>100/stomach) in 6 stomachs and was present in 31 others (percentage occurrence, 40.7).

Plerocercoid Prevalence and Intensity:

Resident Deer Lake rainbow trout were heavily parasitized by Diphyllobothrium plerocercoids (Table 2). The rate of occurrence was 83.8% for the 37 trout examined. The average worm load for parasitized fish was 24 (range 2-55). Encysted plerocercoids predominated (87.3%) in all of the

Table 1. Major zooplanton in the stomachs of coho salmon sampled at Osprey Lake, 1985. Numbers of fish parasitized are in parentheses.

Sample date(s)	Sample source	No. Fish sampled	Percent occurrence of stomach contents: Copepoda: Calanoida	Copepoda: Cyclopoda	Cladocera: Eubosmina	Cladocera: Holopedium	Number of Zooplankters examined for procercoids
10 July	Pen B	10(0)	100	0.0	0.0	0.0	37 Diaptomus
10 July	Pen C	13(0)	84.6	7.7	15.4	7.7	303 Diaptomus
24 July	Pen B	15(15)	100	6.7	73.3	0.0	34 Diaptomus
24 July	Pen C	5(5)	40.0	0.0	80.0	40.0	0
4 August	Pen A	8(8)	62.5	0.0	75.0	12.5	>100 Eubosmina 420 Diaptomus
4 August	Pen C	2(2)	0.0	0.0	100	0.0	0
7 August	Pen C	12(12)	66.7	33.3	25.0	8.3	60 Diaptomus 61 Cyclops
8 August	Pen A	6(6)	83.3	0.0	16.7	16.7	247 Diaptomus
24 August	Pen C	4(4)	0.0	0.0	75.0	0.0	0
7 September	Pen A	3(3)	33.3	0.0	66.7	33.3	118 Diaptomus
7 September	Pen C	2(2)	100	0.0	0.0	0.0	28 Diaptomus
19,22 October	Pen C	15(15)	73.3	6.7	26.7	0.0	462 Diaptomus
Totals	Pen A	17	64.7	0.0	52.9	17.6	785 Diaptomus >100 Eubosmina
	Pen B	25	100	4.0	44.0	0.0	71 Diaptomus
	Pen C	53	69.4	12.2	36.7	8.2	853 Diaptomus 61 Cyclops
Grand Totals		95	73.7	7.7	40.7	7.7	1,709 Diaptomus >100 Eubosmina
Parasitized fish		72	68.1	8.8	51.3	8.8	1,369 Diaptomus 61 Cyclops

Table 2. Prevalences and mean intensities of infestation with Diphyllobothrium plerocercoids for several age classes of rainbow trout captured from Deer Lake between 24 April and 24 July 1985.

Age	Number of fish		Prevalence (%)	Mean intensity of infestation (range)	Total number of plerocercoids	
	Examined	Parasitized			Loose	Encysted
1+	1	0	0.0	-	-	-
2+	25	22	88.0	20 (4-37)	81	353
3+	6	5	3.3	26 (2-52)	2	129
4+	4	3	75.0	35 (24-48)	1	104
5+	1	1	100.0	55	8	47
Totals	37	31	83.8	24 (2-55)	92	633

parasitized trout. The highest ratio of loose/encysted plerocercoids occurred in trout of the 2^+-age class accounting for 88.0% of the loose worms found. Encysted plerocercoid lengths ranged from 4-17 mm. New infestations, denoted by the presence of small worms (usually $<$ 2 mm long) in the stomach wall or near the stomach, were not observed in any of the trout sampled.

In comparison, plerocercoid cysts were not seen in Deer Lake coho until 21 November, 143 days post-stocking. On this date 68.4% of the 19 parasitized coho examined contained cysts and 41.3% of the plerocercoids found were encysted. Plerocercoids were first detected 31 days post-stocking. A peak parasite prevalence of 68.4% occurred in a sample of smolts collected on 24 May, 1986. The average worm load for parasitized fish was 2 (range 1-6). The average worm length increased from 1.6 mm on 2 August to 7.1 mm on 21 November (range 0.75-11 mm). New infestations were not seen after 8 September. Forty-eight coho captured between 2 August and 8 September near mew gull nests (stations D_1 and D_2) contained 75% of all of the plerocercoids in samples of Deer Lake coho. However, it remains unclear whether or not mew gulls disperse diphyllobothriids in Deer Lake. Moribund or dead coho were not observed in Deer Lake.

Diphyllobothrium parasitism in Banner Lake chinook was almost nonexistent (Table 3). Only two chinook with one plerocercoid each were found. The first plerocercoid was detected 40 days post-stocking.

Table 3. Prevalences and mean intensities of infestation of Diphyllobothrium plerocercoids in transplanted coho and chinook salmon sampled at Deer and Banner lakes, 1985 and 1986.

Sample date(s)	Number of fish Examined	Number of fish Parasitized	Prevalence (%)	Mean intensity of infestation (range)
		Deer Lake (Coho)[a]		
1985				
8 July	30	0	0.0	0
19 July	30	0	0.0	0
2 August	25	3	12.0	3 (2-4)
25 August	30	13	43.3	1 (1-3)
8 September	30	8	26.7	2 (1-4)
21 November	35	19	54.3	2 (1-5)
1986				
24 May	19	13	68.4	2 (1-6)
		Banner Lake (Chinook)[b]		
1985				
21 July	30	0	0.0	0
5,6 August	30	0	0.0	0
27 August	30	1	3.3	1
9 September	30	1	3.3	1

[a] fish planted 1-2 July, 1985
[b] fish planted 10 July, 1985

Coho salmon stocked in Osprey Lake in 1975 grew at a faster rate than net pen coho stocked in 1985 (Figure 3). Net pen coho in Osprey Lake grew at a similar rate to Deer Lake coho. The daily growth rate of these coho in 1975 was 1.0 mm during the period 15 July to 12 August (Crone 1981). In comparison, the daily growth rate of coho in net pens from 4 July to 8 August was 0.5 mm. Differences in growth were also observed among the net pen coho. Nonmoribund coho sampled between 4 August and 8 August were highly significantly longer (mean = 67.1 mm) in Pen A, than those sampled from Pen C (mean = 59.3 mm) (t-test, $p < 0.001$).

Diphyllobothrium plerocercoids were first detected in net pen coho 20 days post-stocking. Plerocercoids occurred in 81.2 - 100% of the coho in each sample examined after 10 July (Table 4). The occurrence of plerocercoids in coho was independent of net pen location for each sample date from 10 July and for the total sample period 10 July to 23 October (G-test of independence, $p = 0.05$) Therefore, replicates in the nonmoribund, moribund, and mortality groups were pooled for each pen.

Pen A coho had highly significantly more plerocercoids than Pen C coho in each of the three categories: nonmoribund, moribund, and mortality (Wilcoxon two-sample tests, $p < 0.005$) (Table 5). Moribund and dead coho each had highly significantly larger worm loads than nonmoribund coho (Wilcoxon two-sampled tests, $p < 0.002$), but worm loads of moribund and

Figure 3: Growth of coho salmon in Osprey and Deer lakes.

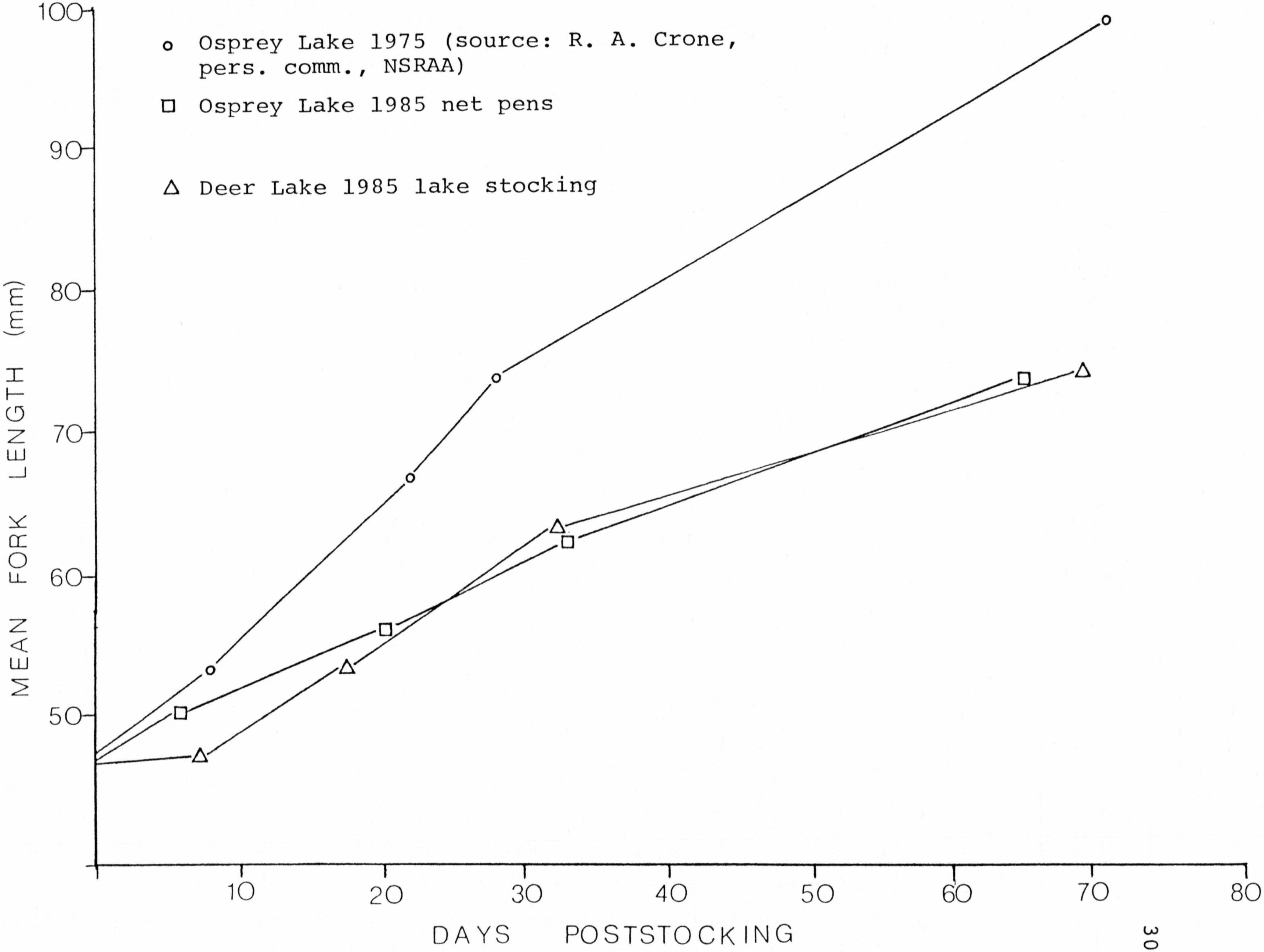

o Osprey Lake 1975 (source: R. A. Crone, pers. comm., NSRAA)
□ Osprey Lake 1985 net pens
△ Deer Lake 1985 lake stocking
MEAN FORK LENGTH (mm)
100
90
80
70
60
50
10
20
30
40
50
60
70
80
DAYS POSTSTOCKING

Table 4. Prevalences and mean intensities of infestation of Diphyllobothrium plerocercoids in nonmoribund and moribund coho salmon sampled from net pens at Osprey Lake, 1985.

Pen	Sample date(s)		Number of fish[a]		Prevalence (%)	Mean intensity of infestation (range)
			Examined	Parasitized		
B	10	July	20	0	0	0
C	10	July	14	0	0	0
B	24	July	18	18	100	8 (2-26)
C	24	July	16	13	81.2	4 (1-8)
A	4,6,8	August	26[b]	26	100	21 (4-44)
C	4,7	August	27	26	96.3	9 (3-25)
A	23,24	August	4	4	100	37 (18-45)
C	23,24	August	9	9	100	12 (2-28)
A	7	September	10	10	100	31 (2-55)
C	7	September	8	8	100	25 (10-41)
C	19,21-23	October	96[c]	96	100	19 (1-55)

[a] Fish transplanted on 4 July, 1985.
[b] Two of these fish had acute ascites.
[c] Five of these fish had acute ascites and were preserved whole for later histopathological examination.

Table 5. Mean intensities of infestation by Diphyllobothrium plerocercoids in nonmoribund, moribund, and dead coho salmon sampled from net pens in Osprey Lake.

Sample date(s)	Net pen	No. fish sampled	Mean intensity of infestation	Range
Nonmoribund				
4 August-7 September	A	18	15.44 ± 10.58	2-41
24 July	B	18	8.39 ± 5.62	2-26
24 July-23 October	C	99	11.12 ± 8.52	1-55
	Total	135	11.33 ± 8.71	1-55
Moribund				
4 August-7 September	A	20	34.70 ± 11.73	15-55
24 August-23 October	C	48	24.71 ± 9.15	8-48
	Total	68	27.50 ± 10.90	8-55
Mortalities				
6 August-7 September	A	20	38.30 ± 15.14	13-68
23 August-22 October	C	12	20.67 ± 8.80	12-41
	Total	32	31.69 ± 15.66	12-68
Moribund and Mortalities				
	Total	100	28.84 ± 12.72	8-68

dead coho were not significantly different (Wilcoxon two-sample tests, $p = 0.05$). Furthermore, worm loads of moribund and dead coho did not significantly change with time (Wilcoxon two-sample tests, $p = 0.05$).

Mortality first occurred on 24 July in Pen A (20 days post-stocking) and on 4 August in Pen C (31 days post-stocking) (Figure 4). Total mortality was 46.6% and 22.4% in Pens A and C, respectively; for an average of 34.5%. Worm loads of mortalities averaged 38 (range 13-68) for Pen A and 21 (range 12-41) for Pen C.

Ascites was first detected on 4 August in Pen A and on 24 August in Pen C. A total of 90 fish sampled from Pens A and C had ascites. In several of these fish it was quite extreme (Figure 5). Sanguineous ascitic fluid was observed in 6 fish at necropsy.

The tissue locations of plerocercoids in 235 coho sampled from the three net pens are listed in Table 6. Most plerocercoids were found loose in the body cavity, especially in moribund and dead fish. The prevalence of plerocercoids in the body cavity and liver was significantly greater in moribund and dead fish than in nonmoribund fish (two-tailed tests where $P_1 = P_2$, $0.001 < p < 0.005$ and $0.010 < p < 0.025$). Parasitized livers of moribund and dead coho averaged two plerocercoids (range 1-6) compared to an average of one (range 1-4) in nonmoribund coho.

Figure 4: Relative frequency of mortality for Osprey Lake coho in Pens A and C.

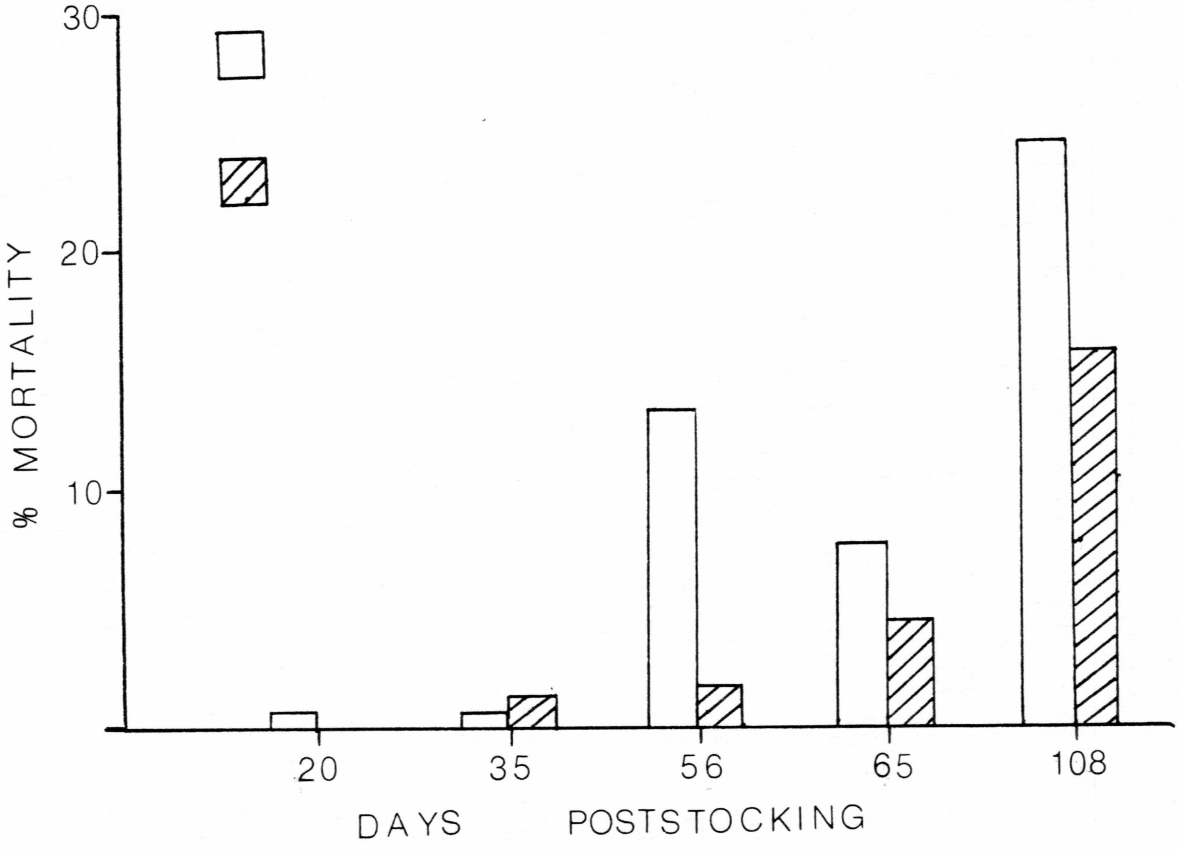

30
20
10
% MORTALITY
20
35
56
65
108
DAYS POSTSTOCKING

Figure 5: Osprey Lake coho with acute ascites.

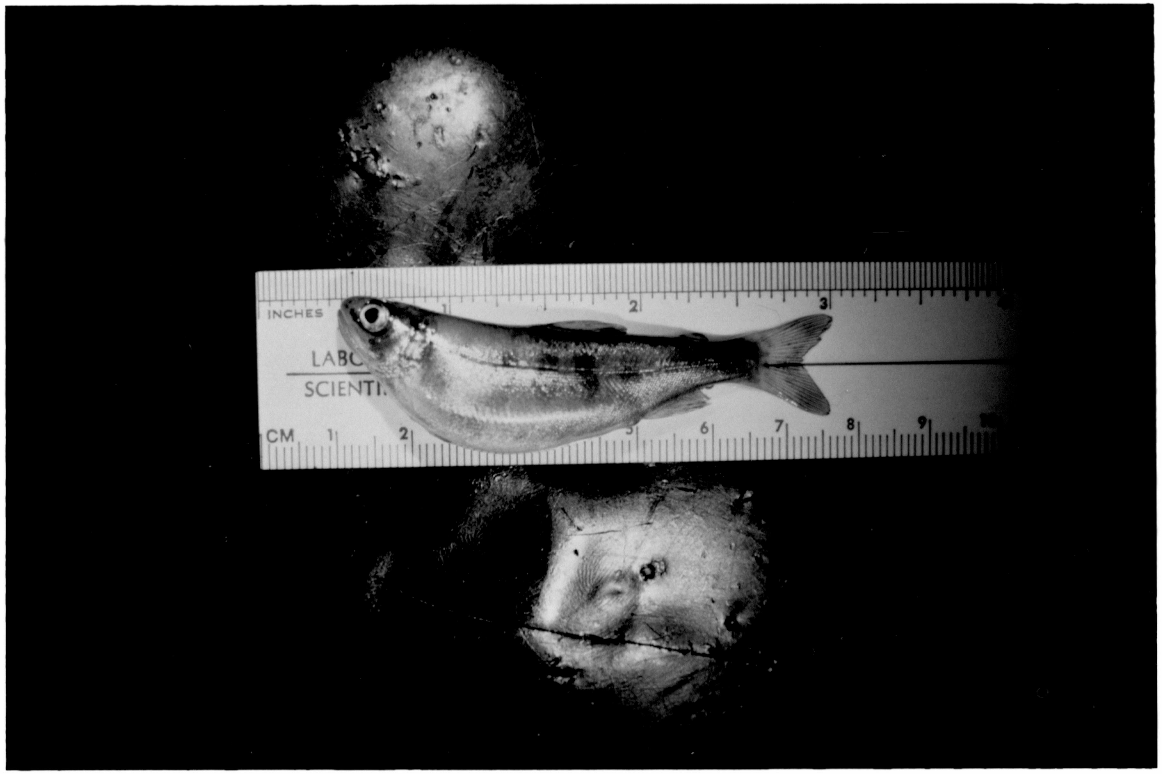

INCHES
CM

Table 6. The site of infestation of Diphyllobothrium ditremum plerocercoids in coho salmon sampled at Osprey Lake, 1985.

Site	Nonmoribund fish (n=135) Total percent of plerocercoids found (n=1,594)	Nonmoribund fish (n=135) Fish with plerocercoids at this site (%)	Moribund and dead fish (n=100) Total percent of plerocercoids found (n=2,869)	Moribund and dead fish (n=100) Fish with plerocercoids at this site (%)
Body Cavity	39.4	86.7	62.6	99.0
Stomach wall	29.9	83.0	15.1	82.0
Pyloric Caeca	16.1	47.4	10.1	61.0
Adipose tissue	9.6	39.3	7.0	50.0
Liver	3.6	31.9	3.3	48.0
Muscle	0.5	3.0	0.9	6.0
Spleen	0.0	0.0	0.4	2.0
Kidney	0.2	1.5	0.1	3.0
Gas Bladder	0.6	5.2	0.3	5.0
Gills	0.0	0.0	0.1	2.0
Ovaries	0.2	2.2	0.0	0.0
Heart	0.0	0.0	0.0	1.0

New infestations were observed in net pen coho through 22 October. A peak occurred on 7-8 August when 15.7% of the coho sampled were newly parasitized. In contrast, cysts, which indicated older infestations, were seen in a total of 17 fish and were first detected on 24 July. Ten of the 17 fish with cysts were sampled after 7 September.

Necropsies were preformed on 32 recaptured coho that had escaped from pens A and B. Two of these were not parasitized, whereas 5 had ascites. Nonmoribund coho, escaped from Pen B and recaptured between 3 August and 8 September, were highly significantly longer (mean = 79.1 mm) than nonmoribund fish sampled from Pen A (mean = 67.9) (t-test, $p < 0.001$) during that same period. Worm loads of nonmoribund and moribund recaptured fish were not significantly different than those from Pen A, respectively (Wilcoxon two-sample tests, $p = 0.05$)

The external morphology of approximately 5,400 plerocercoids recovered from salmonids from Deer, Banner and Osprey lakes appeared identical. Characteristics are in best agreement with those given by Andersen et al. (1987) for the species Diphyllobothrium ditremum. Body surfaces were smooth and glistening. Scolices were protracted and bothrial slits eminently visible. Several specimens were sent to Dr. Hilda Lei Ching of Hydra Enterprises LTD, Vancouver, B. C., who identified them as D. ditremum.

Identification of the Adult Worm:

Among the eight birds collected, only the merganser from Deer lake was parasitized by Diphyllobothrium. Seven mature and two immature worms were found in the small intestine. Scolices from 4 of the adult worms were sent to Ms. Mary Ann Bochert of the Geophysical Institute, University of Alaska, Fairbanks for scanning electron photomicroscopy and comparison to material examined by Anderson (1975). A frontal pit, characteristic for the species D. dendriticum and D. latum, was absent in all four scolices. Scolices were lanceolate to almond shaped.

Two adult specimens were stained and mounted for examination. The following characteristics were delineated. Necks were absent and the largest testes were 100 μm in diameter. Total number of segments varied from 145 to 150 per worm. Maximum segment length and width were 1.4 mm and 4.5 mm, respectively. Total lengths were 11.4 and 12.9 cm. These characteristics were compared to those reported by Markowski (1949), Andersen (1972), and Andersen et al. (1987) for several species of Diphyllobothrium and the closest fit was to that of D. ditremum. Specimens were also sent to Dr. Ching who confirmed their identity.

The infection experiments using chickens had negative results. Cestode eggs were never found in fecal material, and no parasites were observed at necropsy.

Discussion

Procercoid Detection:

Few studies have been published regarding the prevalence of *Diphyllobothrium* sp. procercoid parasitism in natural populations of Copepoda. Henricson (1978) examined a total of 5,720 copepod specimens and found none parasitized. Johnson (1975) made semimonthly collections of copepods for a year and checked a total of 17,000 specimens belonging to 11 species. Only in *Cyclops strennus* did he find three specimens out of 200 examined in mid-July that had procercoids. In the present study there were no procercoids found in a total of 15,711 copepods examined.

The only reported high level of occurrence of *Diphyllobothrium* procercoids in natural populations of Copepoda was observed by Guttowa (1963). She reported peak prevalences of 52% and 23%, in *Cyclops strennus* and *Thermocyclops oithonoides*, respectively. Given these results, it would seem likely that parasitism of copepods in nature by *Diphyllobothrium* procercoids usually occurs at a low level.

Essex (1927) and Guttowa (1963) never found parasitized copepods in nature to host more than one procercoid each. This would indicate that a high prevalence of plerocercoids in second intermediate hosts (fish) should result from a high prevalence of procercoids in populations of first intermediate hosts, copepods (Hopkins 1959).

Rosen and Dick (1983a) supported this idea, as they concluded from experiments with the pseudophyllidean cestode Triaenophorus crassus, that less crowded infestations of Cyclops resulted in a higher percentage recovery of plerocercoids in coregonid fish. In light of these observations, it is surprising that in Osprey Lake, where the peak prevalence of plerocercoids in coho was 100%, there were still no procercoids found in a total of 5,194 copepods examined

Based upon an established peak seasonal density of 40,000 D. kenai per square meter in Osprey Lake there should have been 640,000 D. kenai per net pen or approximately 2,900 per coho. If each coho became parasitized with 19 plerocercoids (the weighted average observed in net pen coho) assuming an 80% transmission rate of procercoid to fish, as reported by others (Miller 1943; Meyer and Vik 1963; and Bylund 1973), then a procercoid prevalence in D. kenai would be approximately 0.83%. This means that only 43 copepods of the 5,194 examined would have been parasitized. This low level of occurrence could explain my failure to find procercoids.

There may have been an additional problem in observing procercoids within copepod hosts. Soon after a coracidium is ingested by a copepod it sheds its ciliated covering, penetrates the gut wall, and takes up residence in the hemocoel (Chandler and Clark 1961). The larval parasite elongates and may reach a size of 500 μm. Sometimes, however, a procercoid may not develop fully, as was the case reported by Miller (1943) for T. crassus and T. nodulosus parasitizing Diaptomus ashlandi. The procercoid may remain as a small sphere of 60 μm or less. Such a shape and size is very similar to that of numerous oil droplets located within the body of D. kenai making procercoid detection difficult, if not impossible. Photographs of small spherical procercoids in Diaptomus gracilis were presented by Duguid and Sheppard (1944). They are very similar in appearance to oil droplets in Cyclops strennus photographed by Guttowa (1963).

Finally, the overdispersion of the distribution of parasite numbers within a host population (Anderson and Gordon 1982) together with sampling error present a third explanation for the difficulty in observing procercoids in copepods. Clumping of parasitized copepods could result from masses of eggs being distributed in lumps of feces. Surface water to a depth of 1 m was sampled with oblique tows of a plankton net in order to concentrate copepods. It was noted by Miller (1943) that Cyclops heavily parasitized (averaging 10 procercoids per Cyclops) with either T. crassus or T. nodulosus tended to settle to the bottom of culture dishes. If parasitized copepods in nature tend to sink, then it is possible they may have been missed by my sampling technique.

Heavily parasitized coho held in net pens in Osprey Lake were restricted to the upper 5.5 m of the water column. On 10 July three vertical zooplankton tows made from that depth near Pen C collected 141 copepods examined for procercoids. Although no procercoids were found this was not a sufficient sample size for parasite detection at a prevalence rate of 0.83% (Simon and Schill 1984). However, a more adequate sample of 1,770 copepods from the stomachs of these net pen coho still did not produce a parasitized planktor. If the efficiency of my methods for detecting procercoids was less than 20%, then a sample of 1770 copepods would also not have been of sufficient size to detect at least one parasitized specimen at the 0.83% rate of occurrence.

Further study is needed to determine whether there are spatial differences between nonparasitized and parasitized copepods. Presently, the low level of copepod parasitism in nature warrants that procercoid prevalence in copepods not be used to assess lakes for potential cestode problems.

The food habits of coho fry in Osprey Lake were intensively studied by Crone (1981). He reported that Diaptomus was the dominant food item present in the stomachs of young coho sampled between 15 July and 26 August 1975. In my study, the abundance of Diaptomus in the stomachs of net pen coho is meaningful because this copepod is a common first intermediate host for diphyllobothriid larvae (Chandler and Clark 1961; Vik 1964; Dau and Barrett 1981). However, empirical evidence is still needed to determine the suitability of D. kenai as a host for Diphyllobothrium procercoids.

Salmon Mortality:

In Banner, Deer, and Osprey lakes the prevalences and intensities of Diphyllobothrium ditremum plerocercoids in stocked salmon were markedly different. Banner Lake chinook were almost free of plerocercoids, Deer Lake coho contracted only light infestations, and Osprey Lake coho were heavily parasitized (Tables 3 and 4). This was the second successive year that Osprey Lake fish sustained a Diphyllobothrium problem. Transplanted chinook salmon in 1984 and coho salmon held in net pens in 1985 sustained numerous Diphyllobothrium mortalities. Among the released chinook fry less than 18% survived to emigrate from the lake (R. Heintz, pers. comm., NMFS). Expected outmigration in a successful year is represented by 58% smolt survival of coho stocked in Osprey Lake in 1975 (Crone 1981). By extrapolation, the chinook mortality due to Diphyllobothrium parasitism could be placed at about 40%.

In the present study, Diphyllobothrium-induced mortality of coho salmon in Osprey Lake was approximately 34%. This figure may be conservative due to the protection of parasitized fish from predation afforded by the net pen enclosures.

The effects of worm load on fish mortality has been debated in the literature. Croften (1971a, b) supported the idea of a lethal threshold level. Anderson and Gordon (1982) argued the relationship in more general terms, "the probability of a host dying in a given time interval is some function of its parasite burden."

The probability of a host dying maybe a function of parasite behavior, since one or two active plerocercoids in a vital organ such as the heart (Bylund 1972) or the brain (Rosen and Dick 1984) could kill a host fish.

Plerocercoid behavior could vary as a result of several interrelated factors, including: plerocercoid migrational ability, number of plerocercoids per fish, host reaction to plerocercoids, and water temperature. For example, higher water temperatures accelerate plerocercoid development (Becker and Brunson 1967) and larger plerocercoids are reported to have better migrational abilities (Halvorsen 1970; Halvorsen and Wissler 1973). Plerocercoids that migrate more can do more damage to host tissues. Bylund (1972) and Rosen and Dick (1983b) reported that the movements of the plerocercoids of D. dendriticum and T. crassus, respectively, were associated with mortalities of coregonid fish. Damage to host tissues may also be greater when there are more plerocercoids involved. However, a dense population of worms within a host might impair plerocercoid growth and consequently limit the ability to migrate. Such tradeoffs confound the concept of a lethal level and may have given rise to the wide variation of worm loads that I observed in dead coho salmon. Plerocercoids within heavily parasitized dead fish (>28 plerocercoids per fish) were highly significantly shorter (mean = 4.47 mm) than those from fish with lesser worm numbers (mean = 5.92 mm) (t-test, $p < 0.001$).

Hosts may prevent further migration of plerocercoids by encapsulation with a layer of connective tissue. The ability to form cysts varies with the host species and in salmonids, encystment does not appear to be well developed (Powell and Chubb 1966; Bylund 1972; Hoffmann et al. 1986). Cyst formation may require several weeks after infestation and then only a few worms may be encapsulated. If plerocercoids are rapidly accumulated, salmonids are defenseless and mortalities will likely occur. Numerous chinook and coho mortalities did occur within two months after stocking Osprey Lake in 1984 and in 1985 (net pens), respectively.

Prestocking Lake Assessment:

A lake may be evaluated for potential salmonid rearing problems due to D. ditremum by using salmon fry in net pens as biological indicators. Several pens smaller than those used in the present study could be evenly distributed in a lake and monitored for a period of at least two months beginning around the first of July. A 4.5-mm mesh, 2-m x 2.5-m x 3.5-m deep pen holding approximately 40 (0.42 g) salmon fry would be adequate and is equal to the stocking density used in my study. This smaller size pen could be handled more easily by one person and the larger mesh size should allow more entry of natural food. Approximately 3 weeks after stocking the pens the presence of plerocercoids could be determined by examining a sample of fish from each pen. During the next 5 weeks pens should be checked frequently for dead and dying fingerlings and these fish should be removed for immediate necropsy. At the end of two months all of the remaining fish would be examined. Observations on plerocercoid prevalence, intensity, location, and size should be recorded as well as fish condition and water temperature. These data will indicate the presence and degree of D. ditremum parasitism. The percent of cestode related mortality is best estimated by the observed number of dead and dying fish. Worm loads may be used to estimate mortality if it is believed that the number of dead and dying fish is not accurately represented. For example, if colder than usual water temperatures occur and plerocercoid development is stunted, the result may be that less harm is done to the host. Worm loads could be heavy enough to cause problems later, though few dead or dying fish are observed. In this instance, worm loads might be a better estimate of expected mortality based on this study. On the other hand, if there are many dead and dying fish, but worm loads are light and worms are not particularly large nor located in a vital organ, then some other factor(s) might be suspected in causing mortality. For example, a hatchery related condition like ceroidosis could predispose fry to stress and death. If mass mortalities occur quickly, diagnosis of a cestode problem might not be possible.

In the present investigation worm loads of age 0^+ coho mortalities varied widely, but none among 32 examined had less than 12 plerocercoids. Furthermore, only 5 of 68 moribund coho and 79 of 135 fingerlings that appeared healthy had less than 12 plerocercoids. These figures suggest that an age 0^+ coho with 12 or more D. ditremum plerocercoids has little chance of surviving. This hypothesis needs further testing.

If a worm load of 12 is used as a threshold level of mortality, it is possible that some lakes might be mistakenly rejected as rearing grounds. Because disease is an issue, a conservative approach is desirable. There are no guarantees that a lake that tests safe before it is stocked will be safe afterwards; however, a lake that tests unsafe should not be stocked.

The consequences of future outbreaks of epizootics could be serious. Besides substantial loss to Alaska's economy, there is a chance of promoting disease by infesting animals that feed on dead and dying fish. These animals might not otherwise be exposed to the parasite. An example is the mew gull, Larus canus, which is very abundant in the study area. These gulls utilize freshwater lakes as rearing grounds and usually build their nests on logs or rocks that are surrounded by water. They are not efficient fish catchers, and they have never been documented as natural hosts of D. ditremum. However, members of the genus have been experimentally infested with plerocercoids of D. osmeri (Vik 1964). Diphyllobothrium osmeri according to Halvorsen (1970) and Bylund (1973) is conspecific with D. ditremum. Bylund's (1973) attempts to infest mew gulls with plerocercoids were unsuccessful. Henricson (1978) also failed to infest L. canus with D. ditremum plerocercoids. If L. canus can serve as a final host of D. ditremum, the potential for spreading parasitism in this area would be tremendous. Further research is needed to determine the ability of this gull to host D. ditremum.

Diphyllobothrium plerocercoids may also be transmitted to large piscivorous fish when they eat zooplanktivorous fish that are parasitized (Stunkard 1965; Freeman and Thompson 1969). If this is true for D. ditremum plerocercoids, then the population of rainbow trout at Deer Lake may be at risk as Deer Lake continues to be stocked with coho salmon fry.

A natural definitive host of D. ditremum in the study area was identified as the common merganser. Mergansers were abundant in the early spring after the lakes thawed. At one point 14 were counted at the outlet of Deer Lake, but few are seen on this lake after May. Two to three loons, Gavia stellata, were also observed on each of the lakes studied. Loons also arrived in early spring, but remained through the summer and into the fall. Mergansers can be legally taken during duck hunting season, so some control of this definitive host is possible. Loons and mew gulls, however, are protected under the Federal Migratory Bird Act. The best control measure is prophylaxis: avoid stocking lakes with a Diphyllobothrium problem.

Future research may do well by examining the development of D. ditremum eggs and procercoids and the effects of D. ditremum procercoids on copepods. Diphyllobothriid eggs take longer to develop and hatch in colder water (Hillard 1960; Meyer 1967). Procercoids may be smaller and less differentiated when the number of larvae per host is large (Miller 1943; Meyer and Vik 1963; Rosen and Dick 1983a). Procercoids also tend to develop faster at warmer water temperatures (Dick and Rosen 1982; Bauer and Solomatova 1984) and procercoids that are more developed are more infective (Rosen and Dick 1983a). Therefore, fewer procercoids may be transmitted to fry if lakes can be stocked early. Lakes could be stocked as early as 15 June without adversely effecting the growth of the rearing salmon (R. A. Crone, per.comm., NSRAA). Conversely, if copepods die as a result of procercoid infestation (Rosen and Dick 1983a), then stocking lakes later (after infested copepods have perished) may result in fewer procercoids being transferred to fish. Both hypotheses are credible and should be tested.

Net pens could be used to facilitate testing. If pens can be placed in a lake in late April or early May, and the former hypothesis proves false, it may be possible to assess the lake for a cestode threat in the same year it is going to be stocked.

THE HISTOPATHOLOGY OF DIPHYLLOBOTHRIUM DITREMUM (CREPLIN) PLEROCERCOIDS IN COHO SALMON, ONCORHYNCHUS KISUTCH, (WALBAUM)

Introduction

Previous reports of mass mortality in salmonids have implicated Diphyllobothrium plerocercoids of various species, but none have described plerocercoids of D. ditremum (Creplin) (Simms and Shaw 1931; Duguid and Sheppard 1944; Hickey and Harris 1947; Fraser 1960; Hoffman and Dunbar 1961). Descriptions of the pathogenic effects of diphyllobothriid plerocercoids, primarily D. dendriticum plerocercoids, have been general and at the macroscopic level (Hickey and Harris 1947; Fraser 1960; Hoffman and Dunbar 1961; Bylund 1972). There are no detailed reports describing the histopathology of salmonids dying from epizootics caused by D. ditremum or other diphyllobothriid plerocercoids. This report describes the histopathological changes resulting from plerocercoids in captive and transplanted coho salmon fingerlings (Oncorhynchus kisutch Walbaum) and the probable causes of death.

Materials and Methods

Eight coho salmon collected from a population of transplanted fish sustaining a plerocercoid epizootic in Elfendahl Lake on Chichagof Island, Alaska in August 1983 were fixed in 10% buffered formalin. Six additional coho from a captive population showing clinical signs of plerocercoid parasitism were also collected from Osprey Lake on Baranof Island, Alaska in August and October 1985 and fixed in 70% ethyl alcohol (ETOH). The gross pathology was described from necropsies of 235 of these fish. Samples from all major organs and tissues of the Elfendahl fish were excised for standard histological processing using 4-6 μm thick sections stained in hematoxylin and eosin. The smaller Osprey Lake specimens were processed whole.

Results

Gross Pathology:

The single external clinical sign of disease was a very marked distention of the abdomen resulting from a clear ascites. In acute cases fish struggled to stay upright, but often floated upside down. Internal gross examination showed adhesions, hemorrhaging, and discolorations of visceral tissues, particularly the liver. The amount of adipose tissue in parasitized fish was reduced. Plerocercoids were observed primarily as loose in the body cavity, but also encysted and unencysted within the stomach wall, connective tissues around the pyloric caecae, adipose tissue, and liver. The minimum number of worms causing ascites and fish mortality was 12 (Chapter I).

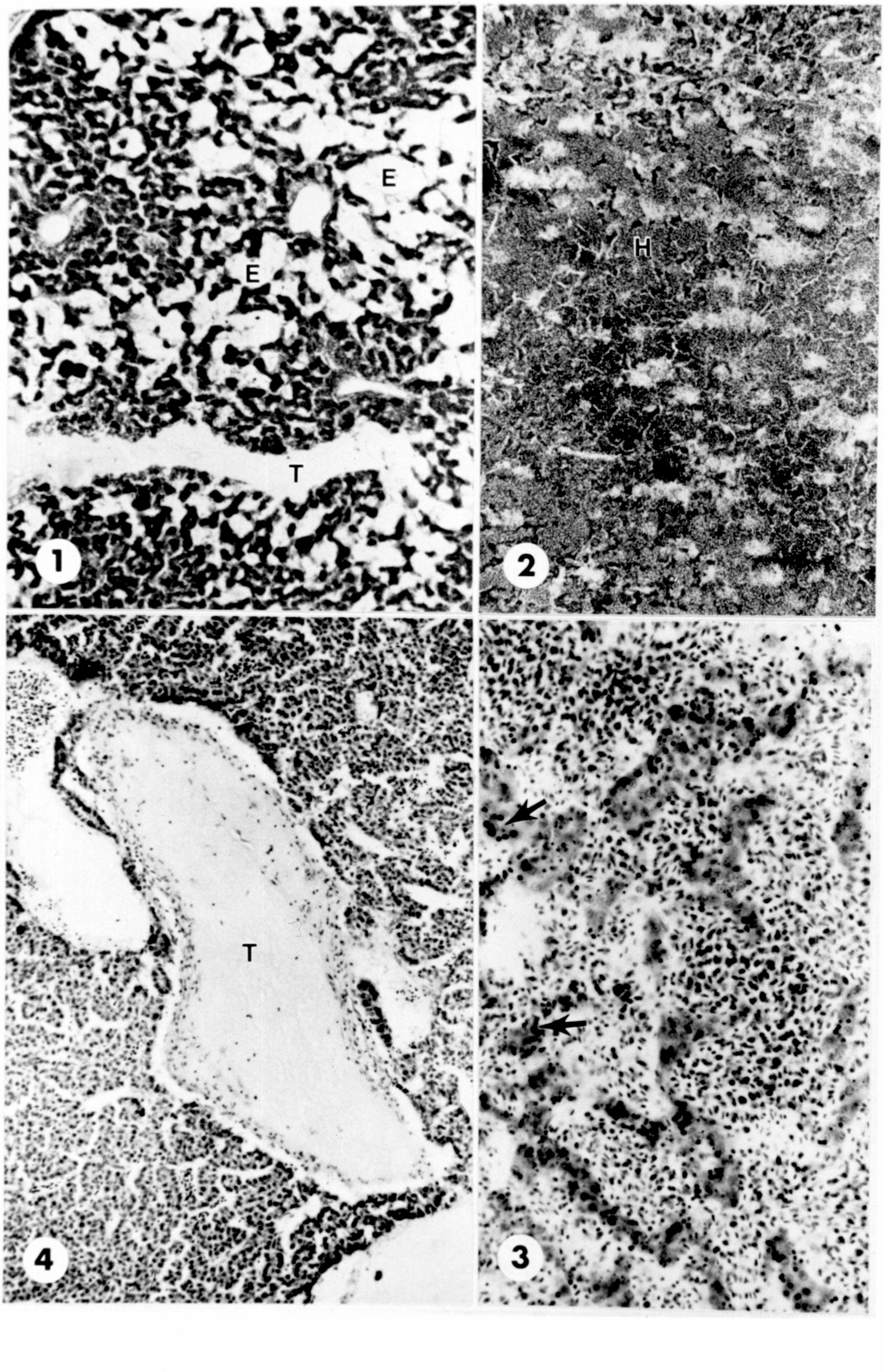
Histopathology:

Hemorrhaging within tissues invaded by plerocercoids was revealed by microscopic examination. Liver was the primary organ affected with edema and congestion of sinusoids causing disruption of the muralial architecture and frank hemorrhaging (Figure 1,2,3). Migrating larvae also left "tracks" of damaged hepatic tissues with little or no fibrosis (Figures 1,4). Host encapsulation of migrating larvae was not pronounced in any of the tissues invaded.

Discussion

Significant fish mortality from D. ditremum plerocercoid parasitism in transplanted juvenile coho (51%) and chinook (40%) salmon and in caged coho fingerlings (34%) has been described in the previous chapter. In parasitized fish the organ most affected and primarily responsible for the major clinical sign of ascites was the liver. Plerocercoid migration damaged hepatic veins obstructing venous flow from the liver causing sinusoidal edema, passive congestion, hepatic necrosis and eventual hemorrhaging within the liver. This condition, also causing the liver discoloration visible grossly, increased intravascular pressure resulting in a transudative edema within the coelom presenting as ascites. Death probably resulted from a combination of liver dysfunction, blood

Figures 1-4. Hematoxylin and eosin-stained paraffin sections of liver from coho salmon fingerlings infested with Diphyllobothrium ditremum plerocercoids. 1. Hepatocyte edema (E) distorting muralial architecture and a plerocercoid "track" (T), x360. 2. Severe congestion and hemorrhage (H) within hepatic sinusoids, x144. 3. Higher magnification of hemorrhagic area in Figure 2 showing hemosiderin-type granules within hepatocytes, x720. 4. Plerocercoid track (T) within normal area of liver filled with fibrin-like material with only slight fibrosis evident around the periphery, x360.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loss and osmotic imbalance. The inability of host fish to contain parasite migration by encapsulation further contributed to their being overwhelmed by relatively few plerocercoid numbers. Indirect mortality from parasitism may also result from predation of compromised host fish unable to equilibrate.

Previous authors considered plerocercoids of D. ditremum to have little effect on the health of fish hosts (Halvorsen 1970; Halvorsen and Wissler 1973). However, the present study clearly demonstrates that small numbers of these plerocercoids can kill significant numbers of fingerling salmonid fishes in experimental and natural circumstances.

CONCLUSIONS

1. A low prevalence of procercoids in naturally occurring copepods precludes use of copepod parasitism as an index for evaluating lakes having a potential problem of Diphyllobothrium parasitism.

2. The use of coho in net pens as a biological indicator is a feasible method of evaluating lakes having a potential problem of Diphyllobothrium parasitism.

3. Plerocercoids of the pseudophyllidean cestode Diphyllobothrium ditremum are significant coho and chinook salmon pathogens. Detrimental lesions observed in coho hosts included: internal hemorrhage of tissues, passive congestion of liver sinusoids, ascites, and adhesions.

4. In caged 0^+-coho salmon at least 12 plerocercoids will result in mortality and at least 8 plerocercoids will cause ascites.

5. Mortality of parasitized caged coho fingerlings is probably less than that which would occur under natural conditions where debilitated fish may easily be captured by a predator.

RECOMMENDATIONS

1. To test a lake for potential <u>Diphyllobothrium</u> parasitism, the use of several 4.5-mm mesh net pens approximately 2-m x 2.5-m x 3.5-m deep with at least 40 salmon fry in each pen is recommended. Pens should be evenly distributed throughout the lake and monitored frequently for 2 months from July through August. Dead and dying fingerlings should be enumerated and necropsies performed on all fish except for decomposing specimens. Fish with 12 or more plerocercoids should be counted as mortalities. Any mortality resulting from the presence of plerocercoids would indicate a lake at risk for a cestode problem.

2. The feasibility of assessing a lake for a cestode threat during the May through June period should be investigated.

3. The rainbow trout at Deer Lake should be monitored for any change in <u>D</u>. <u>ditremum</u> parasitism, should salmon transplants continue there.

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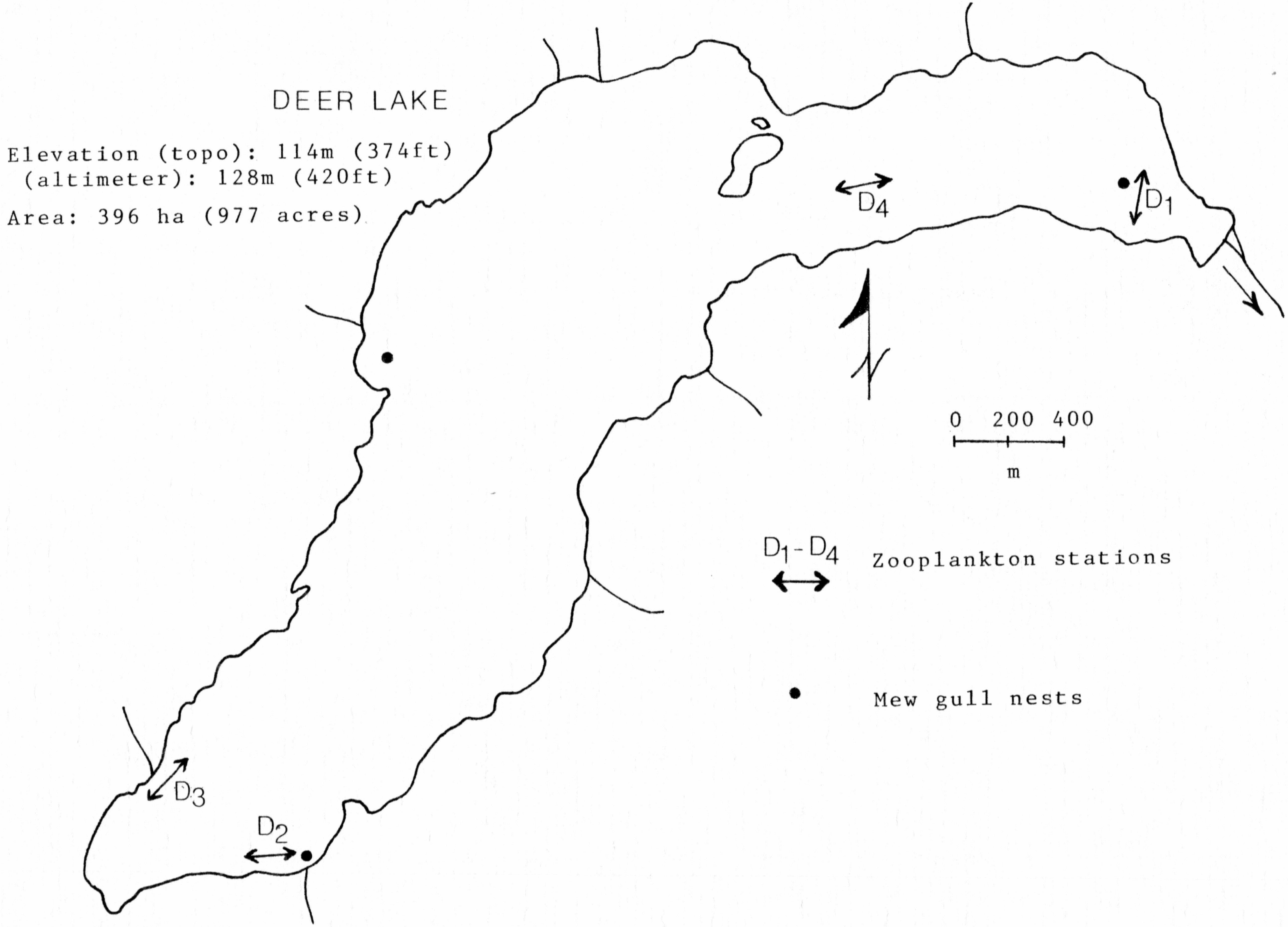
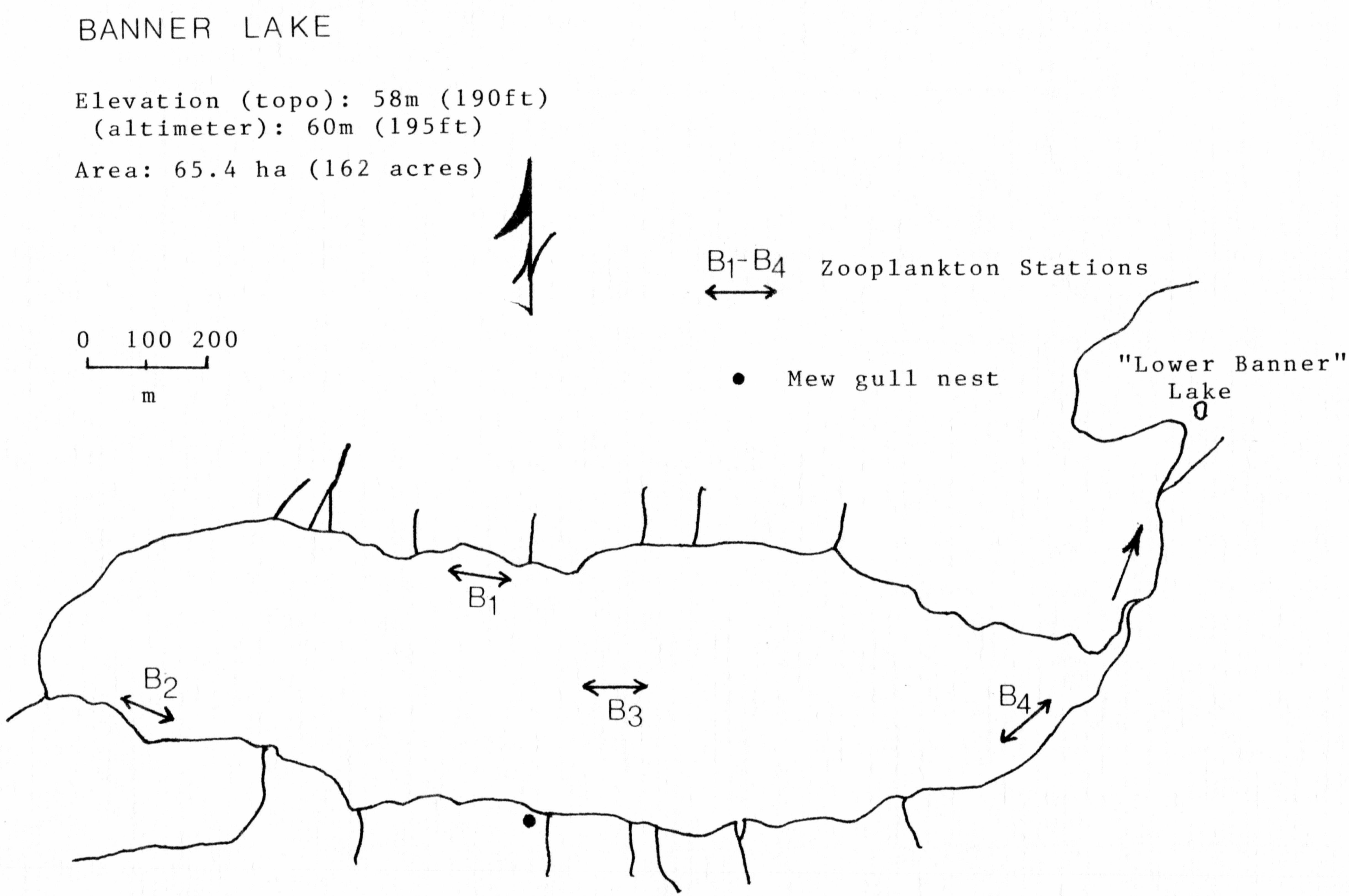
APPENDIX

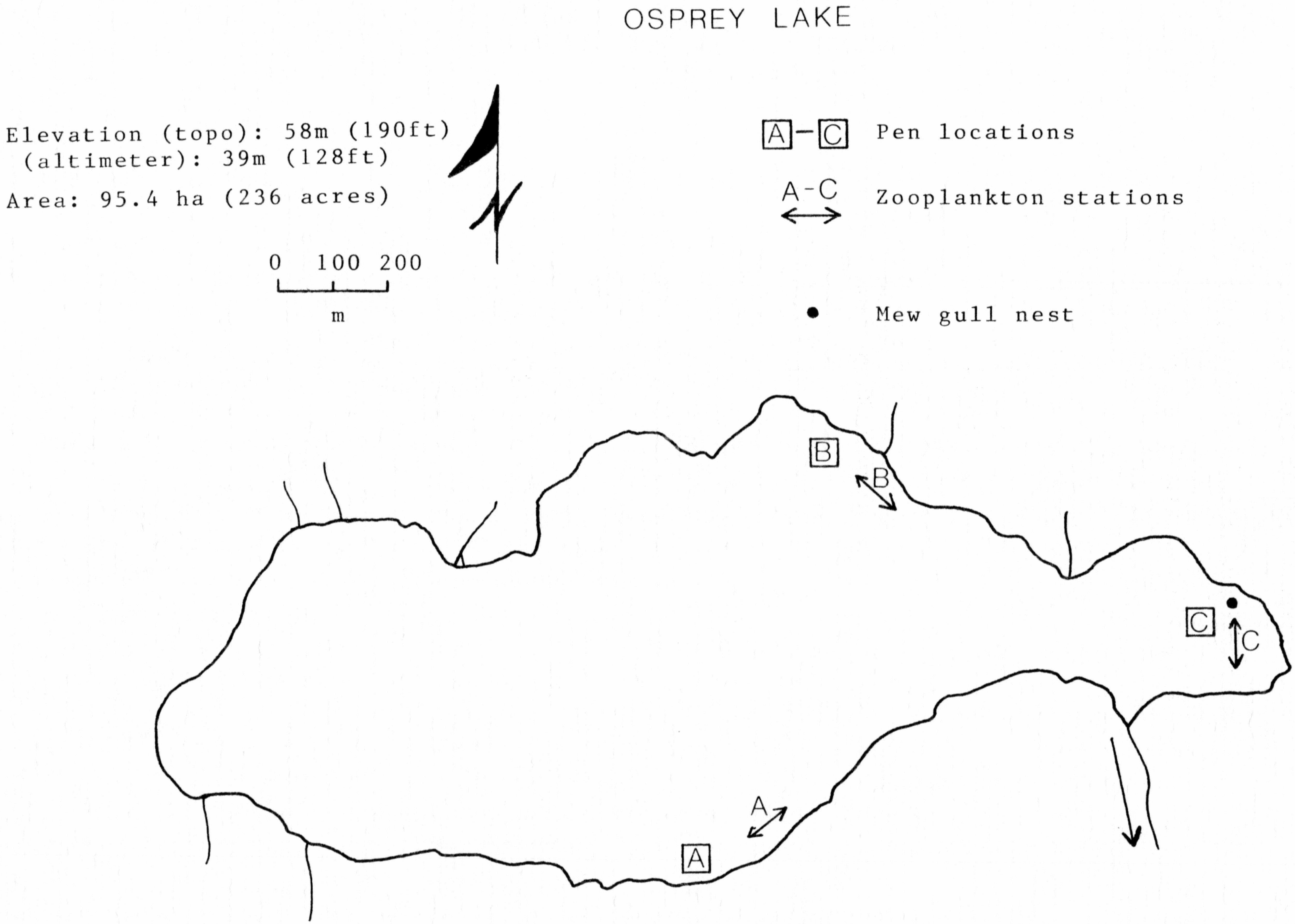
Maps of the three lakes investigated.



Appendix Figure A-1. Location of zooplankton stations in Deer Lake.



Appendix Figure A-2. Location of zooplankton stations in Banner Lake.



Appendix Figure A-3. Location of zooplankton stations and net pens in Osprey Lake.